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IDENTIFICATION OF MICROSPORIDIA (PROTOZOA, MICROSPORA)

Ronny Larsson

Larsson, Ronny: Identifikation of mikrosporidier (Protozoa, Microspora). (Identification of Microsporidia (Protozoa, Microspora)) - Memoranda Soc. Fauna Flora Fennica 59:33-51, 1983.

An identification key is given to the 63 valid genera of microsporidia (Protozoa, Microspora). The key has principally been based on light microscopical characters, which are briefly described. In addition some simple methods for collection and preparation are presented.

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Microsporidia are commonly existing parasitic protozoa which, however, have often been overlooked due to their microscopic size. They can attack all groups of animals, from protozoa to human beings. The most primitive microsporidia are found in the gregarines of the marine bristle worms, while gnats* and mosquitoes are hosts to some of the most specialized. Nosema bombycis Naegeli, 1857, is the oldest named species. It causes pebrine disease which in the middle of the 19th century nearly destroyed the entire world production of silk.

* Transl. note: biting gnats, genus Simulium

Canada

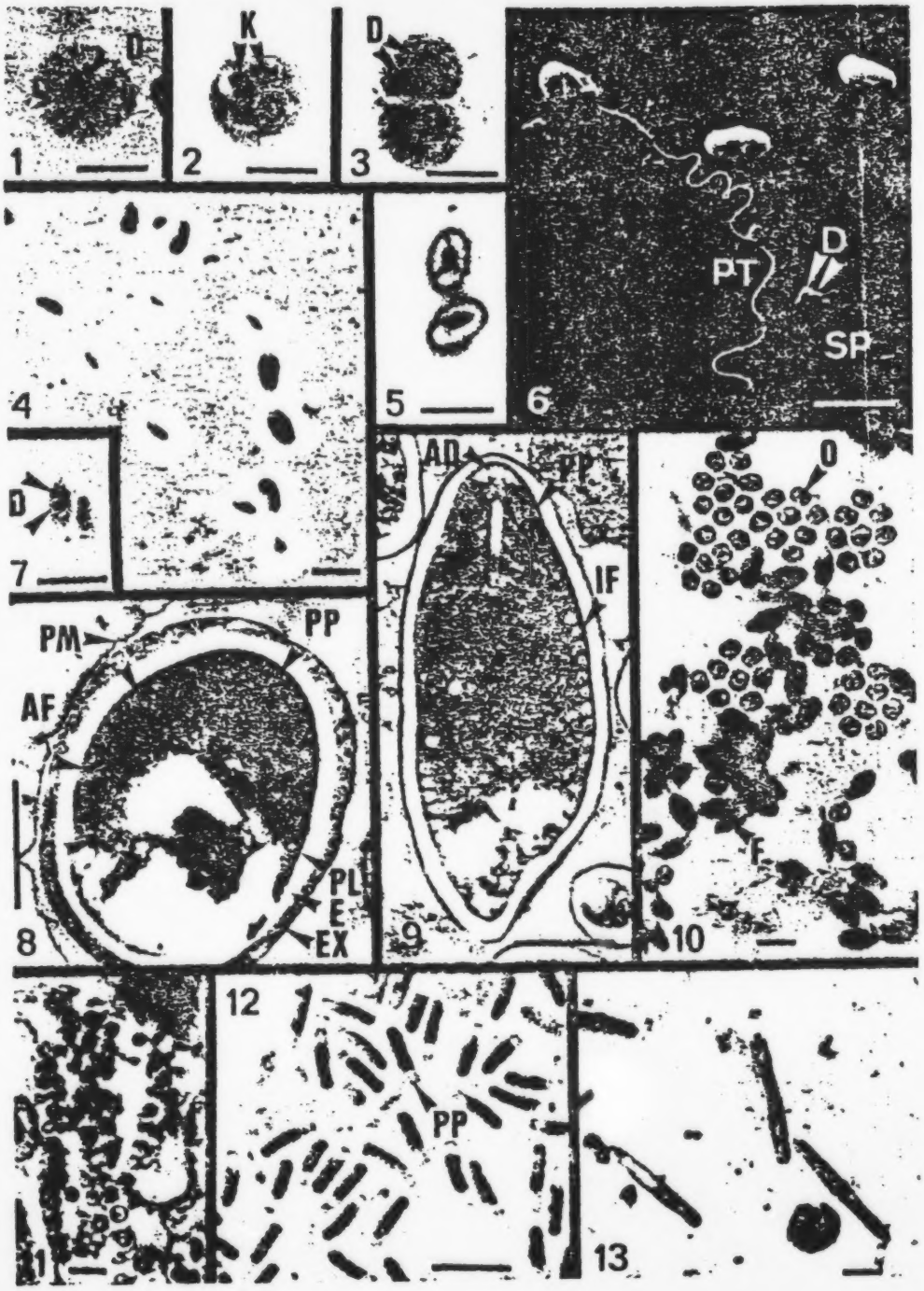
Most well-known is the nosema parasite of the honeybee, N. apis, which exists in all bee-colonies across the entire world. Every bee-keeper is aware of its existence and regularly takes steps to limit the extent of the infection. Another commonly observed microsporidian is Encephalitozoon cuniculi which is regularly reported from laboratory-bred rodents and sometimes from fox farms.

During the last decades, microsporidia have attracted growing international interest since a number of species have proved to be tied to a particular species of host animal. Several microsporidia are already being used for biological control of pests, and plant protection personnel around the world are looking for microsporidia in severe plant pests. The group has also attracted attention within the area of tropical medicine and at the moment, dimorphic microsporidia of the genus Amblyospora are among the most promising of the biological agents for controlling malaria carrying mosquitoes. Hopefully, microsporidia will attract more interest in the future also in Scandinavia. It is not just the areas of plant protection, medicine and veterinary medicine, that may benefit from paying attention to these miniscule parasites. Since animals infected by microsporidia area easily available - one scoop with a dip-net in the nearest pond will normally produce several species of microsporidia - the group offers excellent material for biology instruction, well suited to illustrate various aspects of parasitism in demonstrations and laboratory experiments.

Reports on microsporidia from Scandinavia are few in numbers. This is not due to any lack of microsporidia, but rather to the fact

that there is no easily available literature on identification. No modern work with identification keys exists. The result is that only those who keep up with what is published in protozoological journals are able to identify their findings. The systematics have, however, been dealt with in two large monographies: by Kudo (1924) who lists 170 species distributed among 14 genera, and by Sprague (1977) where 536 species and 45 genera are listed. New species are being described at a rapid pace and in 1981 the total number of species exceeded 600 and the number of valid genera is now 63. Microsporidia in insects were dealt with by Thomson (1960) and Weiser (1961), but both of these works are now nearly obsolete. A modern presentation of the biology can be found in the first volume of the series entitled "Comparative Pathobiology" (1976). Microsporidia have also been discussed in a summary review in the Swedish language (Larsson, 1979). (35)

If a light microscope is available, it is possible in most cases to identify the genus of microsporidia. However, in order to identify the species, examination with an electron microscope is usually necessary. The identification key presented in this article has therefore been limited to genera. For the identification characters of the structure and life cycle of the microsporidia, as well as the relationship between parasite and host are utilized. These are described briefly, followed by simple instructions for collection and preparation.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ABBREVIATIONS USED IN FIGURES

AD	anchoring disc for polar filament	MZ	merozoite
AF	anisofilar polar filament	O	octospore
D	diplokaryon	P	pansporoblast
E	endospore	PL	plasma membrane
EX	exospore	PM	pansporoblast membrane
F	free spore	PP	polaroplast
IF	isofilar polar filament	PT	polar filament
K	nucleus	S	spore
KR	crystal	SG	sporogonial plasmodium
M	meront	SP	sporoplasm
MA	manubrium	SR	sporotn
MS	macrospore		

Figures 1-6. Nosema tractabile: 1. Sporoplasm with diplokaryon; 2. Sporont with 4 nuclei; 3. Two diplokaryotic sporoblasts; 4. Phase-contrast micrograph of unfixed spores; 5. Stained spores; 6. Scanning electron micrograph of spores. The polar filament of one of the spores has been extruded and the diplokaryotic sporoplasm has been released. Fig. 7. Hydrolysed and stained spore with diplokaryon (Nosema mesnili). Figs. 8-10. Amblyospora callosa: 8. Ultrathin section through octospore with anisofilar polar filament; 9. Free spore with isofilar polar filament; 10. Stained smear with octospores and free spores. Fig. 11. Spherical spores of Buxtehudea scaniae. Fig. 12. Rod-shaped spores of Helmichia aggregata. Fig. 13. Rod-shaped spores of Mrazekia sp.

(Scale lines: 1-7, 10, 11-13, 5 µm; 8-9, 1 µm.)

Spore morphology

In an infected host found in nature, the microsporidia usually exist in a sporulated state. The spore is the very resistant transport stage which enables the parasite to travel from one host animal to another without being affected by such external factors as dessication or heat. As their name implies, the spores of microsporidia are small, usually 3-8 µm long (1 mm = 1,000 µm), but in exceptional cases they may reach a length of somewhat more than 20 µm. The shape varies. The cytoplasm is dense, rich in ribosomes and membranes. Mitochondria and contractile vacuoles which are important in free-living protozoa, are always absent in microsporidia. Flagella and cilia are also always absent. The spores are instead equipped with one of the most ingenious devices for infection that exists in the animal world. At the anterior end there is the polaroplast, an organelle composed of lamellae, at the posterior end there is a large vacuole and curled up inside the spore lies the tube-like polar filament, constructed like a telescope and attached to the anterior end of the spore. When the spore has entered a suitable host animal, it is affected by e.g. chemical stimuli, which cause the polaroplast and the vacuole to swell, increasing the internal pressure and the polar filament is ejected at lightning speed. The spore resembles a mine - ready to explode if only given the right start impulse. The polar filament is ejected in the same manner as when a glove is turned inside out. At the same time the telescope is pulled out and the polar filament doubles or triples in

length. Fully ejected the polar filament is 20-30 times longer than the spore. The filament drills into the host cell and through this injection cannula the sporoplasm, the nucleus and cytoplasm of the spore (Fig. 6) can then enter the cell, where further development will take place, and in this way it avoids being damaged by the gastric juices or defense mechanisms of the host.

For the identification of microsporidia, a number of characters related to the structure of the spore are utilized:

The shape of the spore (Figures 4-7, 10-24, 33-46) is often oval or pyriform, pear-shaped. Spherical spores can occur, as well as straight or curved rods. The surface of the spore may have ridges or tail-like appendages (Figures 33-34, 42). One end of the spore may be fanned out like a collar (Fig. 41) or it may be swollen (Fig. 35). Normally, the spores are all of the same size, but in certain genera small spores, microspores, occur together with larger spores, macrospores (Figures 16, 17, 20). (37)

The wall of the spore is usually smooth. Spores that will be dispersed in water are often capable of forming a gel capsule. The wall is tough; it contains e.g. chitin, and in dark-field microscopy it is very refractive (Fig. 15). Its thickness usually varies within an interval of 0.02 - 0.4 μm. In general the spore wall is thinner at the anterior end where the polar filament is anchored. Normally, the spore wall is constructed of three layers (Figures 8,9): the inner plasma membrane, the intermediate layer, the endospore, which may be absent in primitive microsporidia, and the outer layer, the exospore.

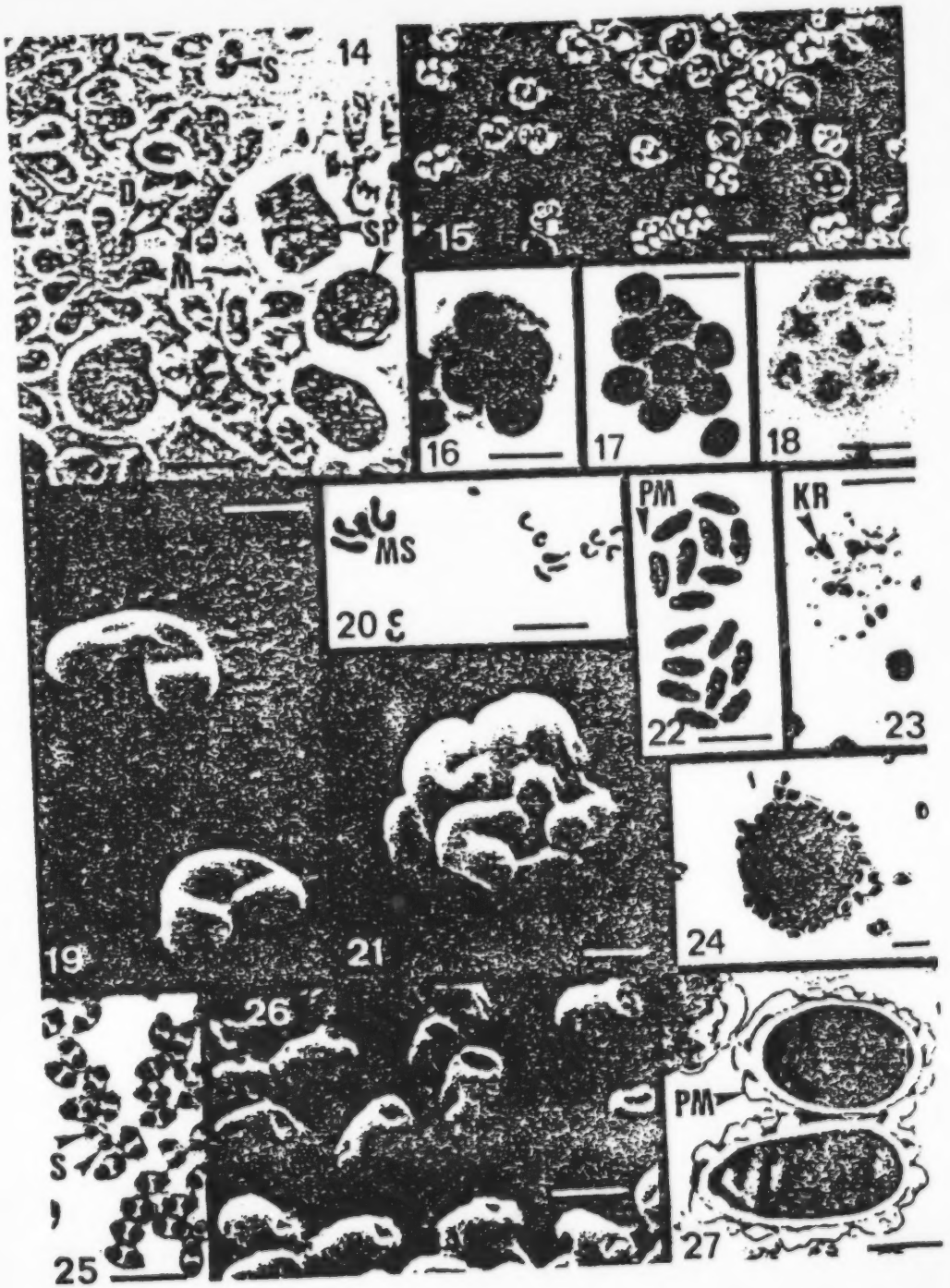
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Figure 14. Stained smear with merogony and sporogony in Amblyospora corethrae.

Fig. 15. Unfixed octospores of Amblyospora bracteata as seen by dark-field microscopy.

Figs. 16-18. Thelohania capillata: 16. Pansporoblast with four macrospores; 17. Pansporoblast with 8 microspores; 18. Hydrolysed pansporoblast with 8 microspores.

Figs. 19-21. Toxoglugea variabilis: 19. Scanning electron micrograph of spores; 20. Stained smear with macro- and microspores; 21. Pansporoblast with 8 microspores as seen by scanning electron microscopy.

Fig. 22. Pansporoblasts of Hyalinocysta sp.

Fig. 23. Pansporoblast with sporoblasts and numerous crystalline inclusions (Amblyospora undulata).

Fig. 24. Pansporoblast of Pleistophora simulii.

Figs. 25-26. Stained smear and scanning electron micrograph of disporous pansporoblasts of Telomyxa glugeiformis.

Fig. 27. Transmission electron micrograph of disporous pansporoblasts of Berwaldia singularis.

(Scale lines: 14-15, 20, 23-25, 10 µm; 16-18, 22, 26, 5 µm; 19, 21, 27, 1 µm.)

The polar filament (Figures 8, 9) emanates from an anchoring disc at the anterior end of the spore. This anchoring disc may appear as a dark-coloured grain in stained smears. The anterior third portion of the polar filament usually extends straight backwards, the rest lies coiled in the posterior end of the spore. The coils may lie in one or two even layers inside the spore wall, or they may be irregularly arranged. The polar filament is usually of even thickness, isofilar (Fig. 9), but there are a number of genera where the anterior portion is thicker than the posterior, anisofilar polar filament (Fig. 8). A small number of genera are characterized by a polar filament whose anterior portion is thick, stiff and straight. It is called manubrium (Fig. 36). In stained smears it is sometimes possible to discern the coils of the polar filament. Any differences in the thickness of the polar filament can be seen when it is extruded.

The polaroplast, which is found at the anterior end of the spore, is usually divided into an anterior and a posterior part (Fig. 9). Both are in general composed of lamellae, arranged concentrically around the polar filament. In the anterior part the lamellae are packed tightly and evenly, but in the posterior part they are arranged more loosely and unevenly. There are also microsporidians where either the anterior or the posterior part of the polaroplast has a cyst-like structure. Even if the structure of the polaroplast cannot be seen in a stained smear, it is still possible to determine its size. Usually, it can be seen as a more lightly coloured area at the anterior end of the spore (Fig. 12).

There is often a vacuole in the posterior end of the spore, and it can sometimes be seen in a stained smear as a colourless area. Certain genera lack a posterior vacuole, or the place of the vacuole may be occupied by a massive structure, the posterosome.

The nucleus is usually located at the centre of the spore. A characteristic of a small number of genera is that their spores contain two nuclei located close together as a diplokaryon (Fig. 7). Also earlier development stages may have diplokarya (Fig. 1-3). In a regular stained smear it may be difficult to separate the nucleus from the cytoplasm. Special DNA stains or acid treatment before regular staining of the nucleus will make the nucleus appear more clearly. In certain cases, the chromatin in a stained smear may give the impression of being binuclear although there is only one nucleus.

LIFE CYCLE

When the sporoplasm has been released (Fig. 6) in one of the cells of the host animal, the reproductive stage will begin. The sporoplasm nucleus divides repeatedly which results in a cell with 2, 4, 8 or perhaps more nuclei. The nuclei are then separated simultaneously, so that each will be surrounded by a thin layer of cytoplasm and an outer protective cell membrane (Fig. 29). This budding process is called merogony, the mother cell is called meront and the liberated daughter cells merozoites. In microsporidia where the merozoites function as the dispersion stage in the body of the host animal, the first merogony is usually followed by one more merogony.

After completed merogonies, the cell walls of the merozoites thicken, and they are then called sporonts. Certain microsporidia do not multiply through merogony; in these the sporoplasm matures directly into a sporont (Fig. 28). The sporont undergoes sporogony and its nucleus divides repeatedly, so that stages with 2, 4, 8 or more nuclei are produced. A multinucleate stage is often called sporogonial plasmodium. There are also microsporidia where spore formation is accomplished through repeated binary fissions. The end products of sporont divisions are called sporoblasts and these mature directly into spores without further divisions. The number of sporoblasts produced from each sporont is usually a characteristic of the genus. (38)

As a rule, both merogony (-ies) and sporogony occur in the life cycle of microsporidia. However, it has not been established that gametes or gametic nuclei are produced. In older descriptions where gamete formation was thought to have been observed, we are most likely dealing with misinterpretations of the stages of divisions. However, it is quite clear that in at least some microsporidia, fusion of nuclei also occurs at some stage of the life cycle. In genera belonging to the family of Thelohaniidae, e.g. Thelohania, Parathelohania and Amblyospora, it has been established that the first division of the sporont is a so-called reduction division, meiosis. This has been confirmed both by light microscopic examinations of stained smears, and by electron microscopy of ultrathin sections where the existence of synaptic complexes, chromosome pairing during the anaphase of the meiosis, have been

observed. If reduction division occurs in the life cycle, there must also exist a process whereby the number of chromosomes in the nucleus are doubled. The only phase of the life cycle which is not well-known is the passage of the sporoplasm through the polar filament at the time of infection. This transport takes quite a long time in most microsporidia, often up to 20 minutes. It is possible that the nucleus divides by mitosis at the time of infection, whereupon the two nuclei immediately fuse, so that the sporoplasm that leaves the polar filament has doubled its set of chromosomes. Another possibility is that the number of chromosomes is doubled without ensuing nuclear division.

A small number of microsporidian species have been found to have two life cycles. In one, groups of mononuclear spores are usually formed, while the other cycle results in free diplokaryotic spores. In Amblyospora species that live as parasites in mosquitoes and gnats* the free spores are found in mature females, whereas the grouped spores are found in larvae. Infected male larvae die before pupation. In female larvae the development of the parasite (39)
is arrested and the larvae can undergo metamorphosis into imagines. Sporogony is then completed in the mature female, the egg is infected in the ovary and in this way the microsporidian is transferred to the next generation of mosquitoes. Infection experiments with spores from larvae have always failed. In A. callosa, a parasite of caddisfly** larvae, the two life cycles occur simultaneously in the larva, and one can find in a smear both large, free, diplokariotic spores and smaller, mononuclear spores in groups of 8 (Fig. 10).

Transl. Note: * biting gnats, genus Simulium
** Order Trichoptera

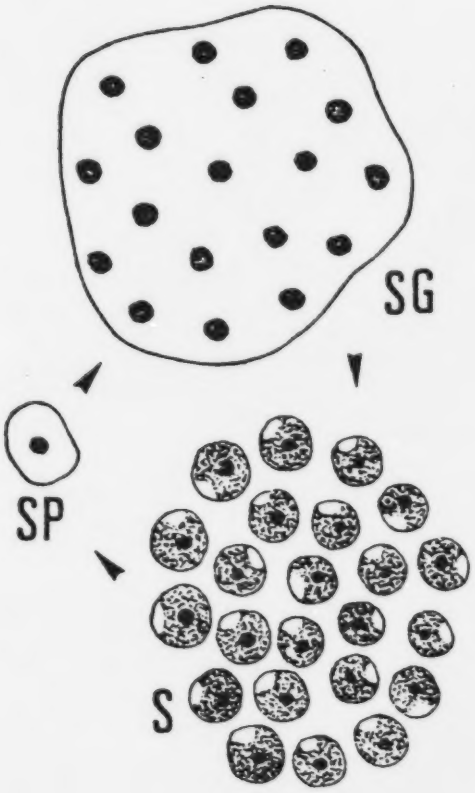


Fig. 28. Life cycle with only sporogony (Buxtehudea scaniae)

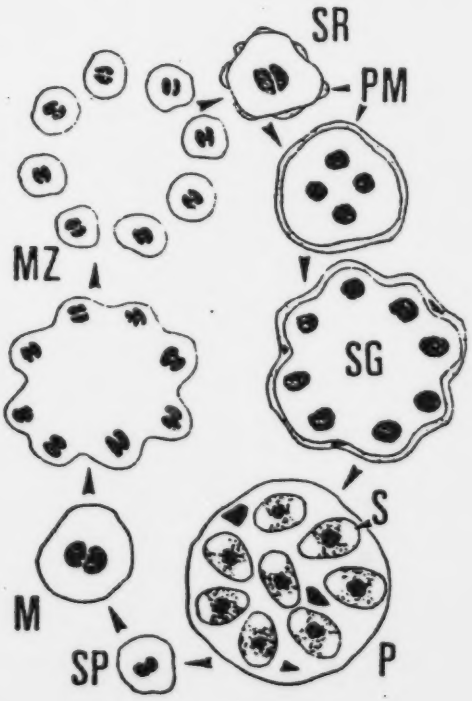
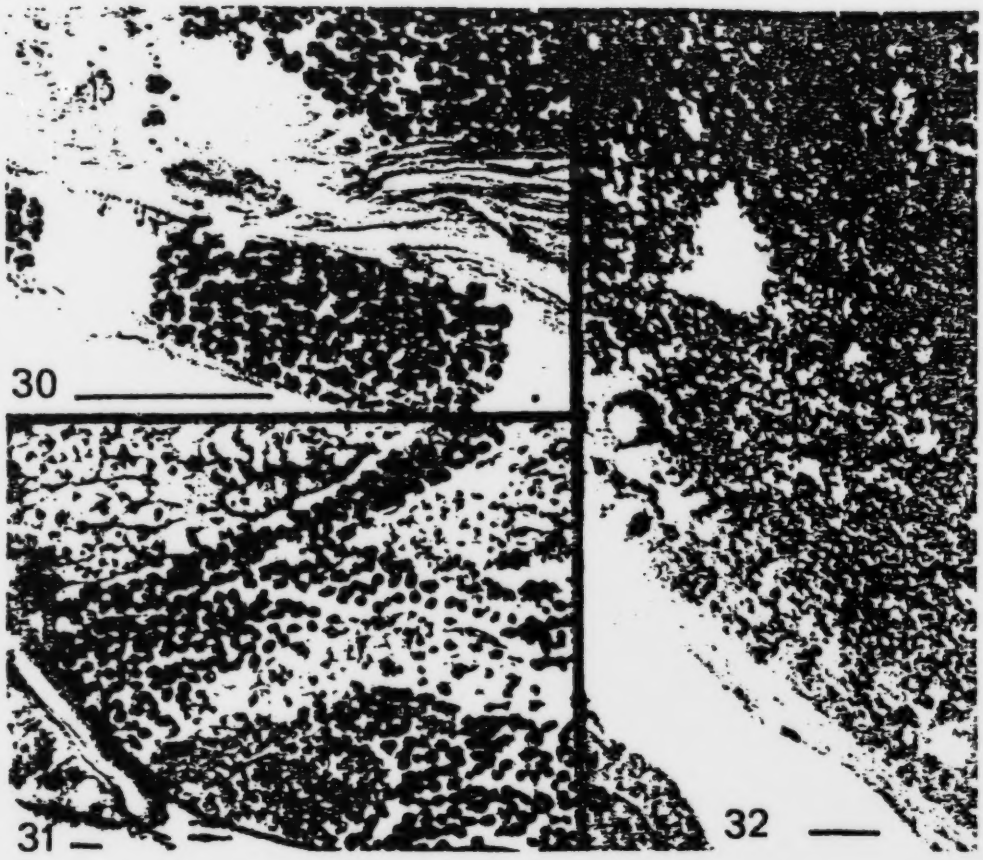


Fig. 29. Life cycle with merogony and sporogony (Amblyospora spp.)



Figs 30-32. Xenoma formation: 30. Syncytial xenoma in Amblyospora undulata; 31. Xenoma with enforced nuclear division of the host cell (Tuzetia debaisieuxi); 32. Xenoma of Glugea anomala with multiplication of host cell nuclei and encapsulating reaction.
(Scale lines: 30, 32, 100 µm; 31, 10 µm.)

INTERRELATION HOST CELL - PARASITE

Since microsporidia always develop intracellularly, the host cell is always affected. The extent of the infection, however, varies between different species of microsporidia, which provides another opportunity to find characters that are useful for identification.

In some cases the parasite develops in such a weak manner that only a few cells are destroyed. This is often the case in the intestinal epithelium. Here, the regeneration ability is also high, so that losses can easily be compensated. However, as a rule all the cells of the infected organ are destroyed. The host cells and their nuclei become enlarged and the organ becomes swollen. In the most extensive infections the organ is transformed into a xenoma, where host cell and parasite fuse into one organic unit. In the most simple cases, the xenoma is produced when the cell walls of the organ are broken down, so that the result is a syncytium, where the nuclei of the host organ and the various stages of the microsporidian swim in a common cytoplasm (Fig. 30). This (40)
is the rule in infections by Amblyospora species. In very severe infections, caused by e.g. Glugea species that parasitize fish (Fig. 32) or by Tuzetia debaisieuxi (Fig. 31), a xenoma is formed from each infected cell. The microsporidian forces the host cell nucleus to divide. The result is a tumour, a dramatically enlarged cell with many host cell nuclei and numerous microsporidia.

Among the microsporidia there exist species that are so strictly tissue specific that they will only attack one tissue in

the host's body. The parasite will then be found only in, for example, fatty tissue or muscular tissue. Other microsporidia cause more extensive infection - in some cases all tissue is destroyed.

Species of the Nosema genus most commonly develop in direct contact with the cytoplasm of the host cell. In other microsporidia the development takes place in a membrane limited vacuole inside the host cell. In numerous microsporidia genera the sporont forms an envelopping membrane, the pansporoblast membrane. Pansporoblast (Figs 16-18, 21-27) is the name given to the membrane with its inclusions. Sporogony is completed inside this envelope. In the space between the membrane and the enclosed cells, crystals are often present (Fig 23) (e.g. in the genera Amblyospora and Cryptosporina), or fibres (Thelohania), or tube-like formations (Toxoglugea). The pansporoblast membrane can rarely be seen in stained smears, while crystals, fibers and enclosed stages of microsporidian development absorb stains. The number of spores that exist within the pansporoblast membrane is usually a characteristic of the genus. If the smears are carefully prepared, spores in groups of 2, 4, 8, 16 or more can be found depending on which genus they belong to. Particularly common are groups of 8 spores. Such spores are called octospores.

COLLECTION

Observable infection

Infection of microsporidia often announces its presence through changes in the appearance and behaviour of the host, or through an abnormally high mortality rate in a population. Infected animals generally move more slowly than healthy. For example, unhealthy water fleas* can sometimes be caught with a pipette. Behavioural changes may cause infected animals to gather in sunny parts of a body of water, while those who are healthy move around the entire mass of water. Aquatic animals that are more or less transparent often become discoloured. They usually become white or pale-yellow, but they can also turn green or red. Infected body parts usually become swollen and particularly in fish, infections of microsporidia can be seen clearly in the form of lumps on the body of the fish, often at the base of fins. In laboratory bred rodents , the first sign of microsporidia infection may be an increase in the number of spontaneous abortions.

Collection of microsporidia

When looking for microsporidia in a population with no visible sign of infection, perhaps in the hope of finding a suitable biological pest control, a great number of individuals must be collected. As a rule the test should include from 100 to 1,000 animals to be ground in a small volume of water, preferably sterile.

* Transl. note: water flea, genus Daphnia

This mixture is strained through a filter cloth in order to remove large tissue particles. The liquid is centrifuged at 1,000 g for 10 minutes, or at a higher number of revolutions for a shorter period. After centrifugation, a clear liquid, supernatant, is found at the top and a lump of sediments at the bottom. One drop of the supernatant is examined in a microscope, but it does not usually contain any microsporidia. The lump of sediments is usually lighter at the bottom and darker on the surface if it containes microsporidia. Most of the spores will be found in the light-coloured bottom layer. When the layers are clearly defined, the bottom material and the surface layer should be separated and then each is diluted with some water. One drop of each suspension is examined in a microscope. If there are no microsporidia spores in the samples, the population is either free from microsporidia, or the rate of infection is very low. In the latter case a new sample with a higher number of animals may reveal the infection. If a small amount of microsporidia are found, it could mean either a weak infection, or that the examined animals ingested microsporidia spores with their food. If microsporidia infection is found, the continued investigation must include a detailed examination of a number of animals from the population through dissection and/or sectioning.

Examination and preparation

In order to identify genera it is normally sufficient to dissect the host animal and make smears of various tissues. However, in

in most cases species identification requires a detailed knowledge of the inner structure of the spore. This requires an electron microscope. A complete examination must include a study of both fresh and fixed/stained material and the following preparations are made of each infected host animal:

A. Smear from infected tissue.
B. A part of the body is fixed and embedded in paraffin wax for sectioning.
C. Some tissue portions are fixed for electron microscopy.
D. One part of the animal is saved.

A. Tissue smears enable a study of the various stages of (41)
the life cycle and are also suitable for morphological observations. But it is not a reliable method for determining which tissues are infected, since it is very difficult to make smears of different tissues without contaminating them. Normally, four different types of smears are made:

1. For direct study fresh smears are placed on cover glasses. A drop of water is added, the cover glass is turned so that the side with the smear faces down and it is then placed on a microscope slide. The preparation is now ready for study. Through this simple method the spores are distributed within the entire amount of liquid and they do not lie still. As a routine, a fresh smear should be photographed, and in order to obtain preparations that can be photographed, a different method can be employed. Liquid

paraffin or agar are used to immobilize the spores. If the cover glass with microsporidia and water drop is placed on a microscope slide with a little liquid paraffin, the water will spread out and the spores will collect in a thin layer immediately underneath the cover glass. This preparation is excellent for study in an ordinary light microscope, but cannot be used for phase-contrast examinations. In the agar method, which is suitable for all types of light microscopes, pretreated slides are normally used. Clean slides are covered with a thin layer of 1 % water solution of agar. The slides are dried in an incubator ($60^{o}C$) and can then be stored in a dry place indefinitely. If the moistened microsporidian smear is placed on an agar slide, the agar will swell and the result is a thin, immobile layer of spores. All fresh smears can be stored in a refrigerator for a few days if the edge of the cover glass is sealed with nail polish to prevent evaporation. Storage for a longer period is not recommended, since it is impossible to prevent the growth of fungi and bacteria in the smears.

2. A small amount of infected tissue is stirred in a drop of water on a microscope slide, and this suspension is then mixed with a drop of Indian ink. In this way gel capsule and spore appendages can be visualized. Bacteriologic ink cannot be used since the ink particles in it are so small that they invade the gel capsule. When the fresh ink smear has been examined, the cover glass can be removed, and after drying the preparation can be fixed and stained.

3. In order to bring about extrusion of the polar filament, tissue smears are made on microscope slides which are left to dry. For identification of species, the length of the polar filament is of interest; for identification of genus it is sometimes necessary to determine whether the filament is iso- or anisofilar. There are a great many different techniques for discharging the polar filament and some microsporidian species require harsher treatment than others. The easiest method is to let the smear air-dry for 30 minutes, then moisten it with water and cover with cover glass. If this does not produce the desired result, the smear can be placed on something hard and the glass be pressed down quite forcefully. Another simple method is to place the smear in a freezer (-20°C) for about 15 min., and then moisten it with water. If these simple methods do not work, various chemicals can be tried. Usually some of the following solutions are used: 5 % hydrogen peroxide, Lugol's iodine solution (iodine, potassium iodide), 1 N sodium hydroxide or potassium hydroxide, or 1 N hydrochloric acid or sulphuric acid. If weak solutions do not work, stronger solutions will have to be tried. In order to get results, the solution should be allowed to work for at least one hour. One can also try to place some crystals of sodium chloride or potassium chloride in the water drop when the smear is moistened. A concentration gradient will be formed around these crystals and this increases the possibilities of creating conditions suitable for the spores.

4. Tissue smears are left to dry until the microsporidia stick, whereupon they are fixed and then stained - for methods, see below. Stained preparations are ideal for studying various stages of the life cycle. They also make it possible to determine whether the spores contain one or two nuclei.

B. Embedding in paraffin and serial sectioning is the only reliable method for studying which tissues have been infected as well as changes in tissue pathology. After the material has been fixed and then washed in 70 % ethanol, embedding can be done in the following simple manner: The tissue specimen is transferred succesively into the solutions listed below. Suitable tempo is one change in the morning, the next in the evening.

a. 96 % ethanol
b. absolute ethanol
c. absolute ethanol/butanol in 1/1 proportions
d. butanol
e. molten paraffin in incubator (60°C)
f. embedding in molten paraffin in forms

Paraffin can be replaced by paraplastic which has a somewhat higher melting point and therefore allows for thinner sectioning. Specimens embedded in paraffin can be stored indefinitely and sectioning can be done whenever it is convenient. Detailed descriptions of the technique can be found in textbooks on histological techniques, such as Baker (1966) or Romeis (1968). The

important part is to know how to fix for sectioning and, if need be, to know how to embed the material in paraffin, so that it can be sectioned, if this type of examination should prove necessary.

C. With regard to <u>electron microscopic examinations</u>, it is also essential to know how to fix the material so that ultrathin sections can be cut, if identification of a species should be desired. Solutions suitable for fixation are listed below. Most of the solutions penetrate the tissue slowly and this is why the tissue specimen must be very small. For those who wish to work with the technique themselves, specialized literature is recommended, e.g. (42) Mercer & Birbeck (1972). Techniques for scanning electron microscopy have been described by, for example, Rausch & Grunewald (1980) and Vávra, Barker & Vivarés (1981).

D. A portion of the infected animal is saved to be used as reference material, or for attempts at infection in order to clarify life cycle and host specificity. Storing in sterile water at refrigerator temperature ($4^{o}C$) will preserve the infectivity of the spores for at least 6 months.

<u>FIXATION</u>

For fixation of smears, or for sectioning, conventional fixatives can be used. However, electron microscopy requires special fixation. The following solutions usually produce satisfactory results:

Formaldehyde, 4-10 % in a water solution often produces reasonably acceptable results. If the solution is neutral, pH 7.0, it can, if necessary, also be used for electron microscope fixation. Both formalin and ethanol are used routinely for zoological conservation, but formalin is preferable as a fixative.

Methanol can be used as a fixative for smears that are to be Giemsa stained. Methanol is poured onto the dry smear and allowed to evaporate.

Picric acid, in the form of an alcohol or water solution, is usually called Bouin's solution in histology. The alcohol solution keeps well, penetrates faster and is easy to use. Two main solutions are prepared which are mixed for use in the ratio: A/B = 2/1. Solution A: 1 g picric acid dissolved in 150 ml of 80 % ethanol. Solution B: 60 ml of 36-40 % formalin and 15 ml crystalline acetic acid. Fixation time: 24 hours - 1 week. After fixation the preparation is washed in 70 % ethanol until the ethanol no longer turns yellow. Material that is to be sectioned is transferred successively up to butanol (see embedding for sectioning), and can then be stored for at least 6 months.

Glutaraldehyde, 2.5-5 % solution, is recommended for electron microscopic fixation. A buffer is used as solvent, e.g. 0.2 M sodium cacodylate, at a pH of 7.0-7.4. Fixation can be done at room temperature, suitable time is 2-4 hours, but fixation in a refrigerator ($4^{o}C$) is preferable. After fixation the material is

washed in a buffer and can then be stored in the buffer at refrigerator temperature for a couple of months.

Osmium tetroxide, 2 % in a buffer solution, e.g. sodium cacodylate, is often used for electron microscopy as a post-fixative to glutaraldehyde. It can also be used alone as a fixative. After fixation the material is washed in a buffer. Storing as for glutaraldehyde fixation.

STAINING

Most histological staining methods that stain nuclei can be used. The following have proved suitable for microsporidia:

Giemsa staining is the most commonly used method. It is easy to do, requires a minimum of chemicals and makes no great demands on fixation. The staining solution, which contains azure-eosin-methylene blue, can be bought ready-made. For staining a 10 % solution in distilled water or in a neutral-slightly alkaline buffer is prepared. The preparation is usually left in the staining solution until it has absorbed enough colour, about 20-30 minutes. Better results are, in general, obtained if the preparation is overstained for about 1 hour and the excess dye is then removed in 70 % ethanol. Smears can be stained using either method, the latter is preferable for sectioning. Both nuclei and other cell structures are stained with this method. Nuclei will stand out better if the preparations

are pretreated with 1 N hydrochloric acid in an incubator (60°C). The exact time needed is a matter of trial and error, 5-15 min. is usually sufficient. The preparations are rinsed with distilled water between the acid treatment and the staining. Acid treatment of smears can also be done in a different way. A small drop of 1 N hydrochloric acid is placed on the dry smear. The glass is then heated in a flame until the drop starts to boil. The glass is then rinsed with distilled water and stained as above. The advantage with this method is that the same glass will contain both untreated and acid treated spores.

Hematoxylin staining is by far the best method for microsporidia. However, it requires more practice than Giemsa staining and it is more time consuming. Type materials should be stained in this way. It is usually done according to Heinehain's recipe. Smears or sections are treated in the following way:

a. The preparation is left in a 2.5 % water solution of iron alum for 3-12 hours.
b. Wash in distilled water.
c. Stain in hematoxylin solution for 5-36 hours, whereby the preparation becomes very overstained. The dye is prepared 4-5 weeks in advance and poured into a bottle. The opening is covered with a cotton plug and the dye is then left to mature at room temperature. After this treatment the dye can be stored nearly indefinitely, if

it is kept in a tightly closed bottle. Recipe: Dissolve 0.5 g hematoxylin in 10 ml of 96 % ethanol and then add 90 ml of distilled water.

d. Remove excess dye in a 2.5 % iron alum solution. Check in a microscope. The nuclei should be black, other tissues nearly colourless.

e. Rinse in running tap water for 10-15 minutes.

Feulgen staining is used to stain the DNA of nuclei and (43)
is often used as a selective nucleus stain. The procedure is as follows:

a. Pretreat preparations in 1 N hydrochloric acid at 60°C for 5-15 minutes.

b. Rinse in distilled water.

c. Stain for 2-3 hours in a solution prepared as follows: Dissolve 1 g basic fuchsin in 200 ml boiling water, then filter and let cool to about 50°C. Then add 20 ml 1 N hydrochloric acid. When the solution has cooled off to room temperature, add 1 g anhydrous sodium bisulphite, whereby the solution is de-coloured. Let the solution cool in a refrigerator. After 24 hours it is ready for use. It can be stored for 2-3 months in a refrigerator in a well sealed bottle.

d. After staining, rinse the preparations in a sulphurous dioxide solution, 3 baths of 2 min. each. The solution

is to be mixed just before use and consists of: 10 ml 1 N hydrochloric acid, 10 ml of 10 % sodium bisulphite solution in distilled water, plus 200 ml distilled water.

e. Rinse in tap water for 5-15 minutes.

MOUNTING

After staining, smears are left to air-dry and are then covered with a neutral, synthetic mounting medium, e.g. DPX plus cover glass. Sections are passed through a series of ethanol solutions of increasing concentration: 70 % - 96 % - absolute ethanol. They are then transferred into xylol and covered with mounting medium and cover glass.

IDENTIFICATION KEY FOR MICROSPORIDIA GENERA

Several genera have been described extremely diffusely and incompletely and they include only one species. These have also been included in the table, but commented on separately. The genus Pleistophora s. lat. presently encompasses a large number of species, both from fish and invertebrates. The type species, P. typicalis, was recently rediscovered and examined with modern methods. The species was given a new description and the genus was more strictly limited (Canning & Nicholas, 1980). In the future, the genus Pleistophora s. str. will probably be reserved for fish parasitic species, while other Pleistophora species will likely be grouped into three new

genera. Some of the microsporidia which are now placed in the genus Tuzetia, were previously considered Pleistophora species, but were removed from this genus when their ultrastructure became known. Sprague (1977) introduced the genus Microsporidium as a gathering genus for species that were so insufficiently known that they could not even be definitely placed in any genus. Since this genus is characterized solely by its heterogeneity and absence of characters, it was, naturally, not included in the table.

The table is based on characteristics that can be observed in an ordinary light microscope. In some cases electron microscopic characters have also been included, either because genus identification requires knowledge of these characters, or because these characters considerably simplify identification.

1. Spores cemented together in pairs in oval, massive pansporoblast (Fig. 25) ..... Telomyxa
- Others 2
2. Spores with clearly defined tail (Figs 33-34)..... 3
- Spores of varying appearance but without clearly defined tail 4
3. Spores: rod-shaped (Fig 33), with 1 nucleus, earthworm parasites ..... Jirovecia
- Spores: oval (Fig. 34), with 2 nuclei, parasites of larvae of biting gnats (Simulium)... Caudospora
4. All spores rod-shaped; cylindrical, straight, curved or spiral-twisted 5

- Spores not rod-shaped or rod-shaped free spores occur together with oval spores 11

5. Spores "c"-shaped or spiral-twisted (Figs 19-21) ..... Toxoglugea

- Spores straight or slightly curved 6

6. Posterior end of spore enlarged (Fig. 35), sporulation in direct contact with the cytoplasm, each sporont forms 2 spores ..... Cougourdella

- Spores without such pronounced swelling 7

7. Spores in groups of 8 spores 8

- Spores free or in multisporous groups 10

8. Spores with 2 nuclei, length 3-10 m ..Octosporea

- Spores with 1 nucleus 9

9. Spore length exceeds 16 µm, parasites of crustaceans; with manubrium, two polar rings in anterior end of spore and spongelike anterior polaroplast region ..... Ormieresia

- Spore length not exceeding 7 µm, insect parasites (Fig. 12); without manubrium and polar rings, both polaroplast regions lamellar .....Helmichia

10. Spores with 1 nucleus, length not exceeding 5 µm, multisporous groups in vacuole, crustacea parasites ..... Baculea

- Spores with 2 nuclei, length normally exceeds 15 µm, not in groups, mainly earthworm parasites (Figs 13, 36)..... Mrazekia

11. Numerous oval spores occur together with numerous rod-shaped spores, all with 1 nucleus, parasites of gregarines ..... Nosemoides
- No spores are rod-shaped 12
12. Spores spherical, one pole may be somewhat flattened (Fig. 11) 13
- Others 22
13. Spores in octosporous pansporoblasts .... Pilosporella
- Spores free or in multisporous pansporoblasts ... 14
14. Parasites of gregarines, spores enclosed both by a vacuole and by a thick-walled pansporoblast membrane, absence of polaroplast and endospore... 15
- Not parasites of gregarines, spores usually not enclosed by a thick-walled envelope, polaroplast and endospore of varying structure 17
15. The ends of the pansporoblast are elongated into points (Fig. 37) ..... Amphiacantha
- The ends of the pansporoblast are rounded 16
16. The pansporoblast is shorter than 10 times the diameter ..... Metchnikovella
- The pansporoblast is longer than 10 times the diameter (Fig. 38) ..... Amphiamblys
17. The parasite develops in the proximity of the nucleus of the host cell (Fig. 39) 18
- The parasite is not found in particular proximity of the nucleus 19

18. Parasite of molluscs, particularly in intestinal epithelium and ovaries, outer envelope of polar filament is a honeycomb layer ..... Steinhausia
- Parasite in intestinal epithelium of insects or polypods (Fig. 39), normal polar filament .... Chytridiopsis
19. The pansporoblast membrane constructed as a mosaic of polygonal plates , non-sporulated stages with diplokarya, parasite in intestinal epithelium of insects ..... Hessea
- Neither pansporoblast membrane nor diplokarya present 20
20. Parasite in musculature or skin of segmented worms, infected cells become enlarged and form xenomas... ..... Burkea
- No xenoma formation 21
21. In intestinal epithelium of insects (Fig. 11).... ..... Buxtehudea
- In intestinal epithelium of segmented worms .... Jiroveciana
22. Spores with 4-5 threadlike, long appendages (Fig. 40), in octosporous pansporoblasts .. Inodosporous
- Spores without long appendages, free or in groups 23
23. The posterior end of each spore fans out like a collar (Fig. 41), in octosporous pansporoblasts .. .. Parathelohania
- Others 24

24. Spores with longitudinal ridges (Fig. 42), double nuclei ..... Weiseria
- Different spores 25
25. Spores with 2 nuclei with flattened sides (Fig. 43) ..... Golbergia
- Others 26
26. Pansporoblast membrane with threadlike appendages 27
- Pansporoblast membrane, if present, is always without appendages 28
27. Pansporoblast round, with (3-)4 long spines (Fig. 44), with 16 spores ..... Trichoduboscqia
- Pansporoblasts with more than 16 spores, pansporoblast spindle-shaped or triangular with corners extended into threads ..... Mitoplistophora
28. Large, free binuclear spores occuring together with smaller mononuclear spores which are most often enclosed in pansporoblasts 29
- All spores are either binuclear or mononuclear .. 34
29. Mononuclear spores in octosporous pansporoblasts . 30
- Mononuclear spores are formed in irregular numbers 33
30. No secreted granules in the pansporoblast, each sporont forms two binuclear spores 31
- Pansporoblast with secreted granules, each sporont forms several binuclear spores 32

31. The pansporoblast membrane is thick, difficult to crush, the polar filament is coiled in one layer close to the spore wall ..... Vairimorpha
- The pansporoblast membrane is thin, breaks easily, coils of polar filament are irregularly arranged ..... Burenella

32. Octospores with anisofilar polar filament (Fig. 8) ..... Amblyospora
- Octospores with isofilar polar filament ..... (46)
..... Thelohania

33. Spore wall covered externally with a thick secretion, resembling sunbeams ..... Auraspora[1)]
- Spore wall of normal structure ..... Hazardia

34. All or some of the spores enclosed in pansporoblasts or in a capsule formed by host tissue 35
- All spores free 60

35. Pansporoblasts with four macrospores occur mixed with free microspores ..... Stempellia[2)]
- Spores in pansporoblasts with 2, 4 or more spores or spores enclosed in a capsule of host tissue .. 36

36. Pansporoblasts with 4, 8 or 16 spores of uniform size occur mixed and in approximately equal numbers ..... Culicospora
- Spore groups with a uniform number of spores (2, 4, 8, 16, 32 or more). Sometimes a small number of pansporoblasts with 4 macrospores may occur together with pansporoblasts with 8 microspores,

or a small number of pansporoblasts with 8 macrospores may occur together with multisporous pansporoblasts with microspores 37

37. Spores connected in pairs 38

- Spores in different groups 40

38. Spores binuclear ..... Issia

- Spores mononuclear 39

39. Merozoites with diplokarya, large pansporoblast, membrane not attached to spores .....Neoperezia

- All stages with single nuclei, pansporoblast membrane attached in folds on the surface of the spores (Fig. 27) ..... Berwaldia

40. Spores in pansporoblasts with 4 spores 41

- Spores in other groups 42

41. Spores drop-shaped with elongated, curved point (Fig. 45), crustacea parasites ..... Pyrotheca[3)]

- Spores pyriform (Fig. 46), parasites of various invertebrates ..... Gurleya

43. Pansporoblasts oval, with numerous large amber-coloured secreted granules which in fresh smears nearly completely conceal the pyriform spores .. .. Cryptosporina

- Pansporoblasts of varying shape, if secreted granules are present, they never conceal the spores . 44

44. Pansporoblasts joined by cytoplasmic filaments until sporulation is completed, pansporoblasts are formed from multinucleate plasmodia... Pegmatheca

- Pansporoblasts free from each other 45

45. Pansporoblasts with elongated spindle-shape, spores pyriform ..... Chapmanium

- Pansporoblasts rounded or oval 46

46. Pansporoblasts without secretion granules 47

- Pansporoblasts with more or less distinct secretion granules, at least in pansporoblasts with immature spores (Fig. 23) 48

47. Pansporoblasts oval, the usually pyriform spores appear clearly against a bright background (Fig. 22), polar filament anisofilar, polaroplast large and lamellar, insect parasites ..... Hyalinocysta

- Pansporoblasts spherical - oval, spores pyriform, the nuclear divisions of the sporont are completed before the cytoplasm begins to divide, polar filament anisofilar, polaroplast unclear, crustacea parasites ..... Agmasoma

48. Polar filament anisofilar 49

- Polar filament isofilar 50

49. Spores pyriform ..... Systenostrema

- Spores with thin oval shape ..... Amblyospora

50. Fish parasites, in exceptions pansporoblasts with 16 spores may occur together with octosporous pansporoblasts ..... Heterosporis[4])

- Invertebrate parasites, in exceptions pansporoblasts with 4 spores may occur together with octosporous pansporoblasts (Figs 16-17) ..... Thelohania

51. Spores in pansporoblasts with 16 spores, pansporoblast with smooth wall without appendages .... Duboscqia

\- Spores in other groupings 52

52. Each spore enclosed in its own pansporoblast membrane, each sporont forms 3-6 spores .... Tuzetia

\- Spores in groups enclosed by pansporoblast membrane or in xenomas encapsulated by the host animal (Fig. 32) 53

53. Fish parasites that form large xenomas 54

\- Parasites of various host animals, spores enclosed by pansporoblast membrane, no xenoma formation ... 56

54. Xenoma unicellular, the nucleus of the host cell becomes enlarged and undergoes divisions, xenoma surrounded by a thick-walled capsule (Fig. 32), spores mononuclear ..... Glugea[5)]

\- Xenoma multicellular, only the cytoplasm of the host cell is attacked 55

55. Xenoma resembling a cluster of grapes in nerve tissue, surrounded by a thick wall, spores usually mononuclear ..... Spraguea[8)]

\- Large xenoma in the connective tissue of the abdomen, surrounded by a single layer of cells, spores binuclear ..... Ichthyosporidium

56. Sporonial plasmodium sends in finger-like projections into the cytoplasm of the host cell, spores numerous, binuclear ..... Pseudopleistophora

- Others, spores with 1 or 2 nuclei 57

57. Each pansporoblast with 5-6 binuclear spores, insect parasites ..... Culicosporella

- Pansporoblasts with numerous, mononuclear spores, parasites of various host animals 58

58. Pansporoblasts with, in general, 32 spores, in exceptions with larger numbers, occasionally both microspores and macrospores may occur, parasites of mosquitoes and biting gnats*..... Vavraia[4)] (47)

- Pansporoblasts with more than 32 spores. Pansporoblasts with 8 macrospores can occur in a small number together with multisporous pansporoblasts . 59

59. Pansporoblasts with more than 32 spores of uniform size (Fig. 24) ..... Pleistophora s. lat.

- Pansporoblasts with more than 32 spores occur together with a small number of octosporous pansporoblasts with macrospores Pleistophora s. str.

60. Spores apparently free, but each spore is actually enclosed by its own pansporoblast membrane; each sporont forms 3-6 spores ..... Tuzetia

- Spores not enclosed in pansporoblast membrane, each sporont produces 2 spores or more than 6 spores .. 61

61. Spores binuclear 62

- Spores mononuclear 64

62. Each sporont produces 2 spores (Figs 2-4). Nosema

- Each sporont produces a larger number of spores .. 63

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* Transl. note: mosquitoes (Culex), biting gnats (Simulium)

63. Parasites of mature female mosquitoes .....
..... Amblyospora[6)]
..... Parathelohania[6)]
- Parasites of crustaceans ..... Ameson

64. Each sporont produces 2 spores 65
- Each sporont produces 6 or more spores, parasites of gregarines 67

65. Parasites of vertebrates ..... Encephalitozoon
- Parasites of invertebrates 66

66. Parasites of trematodes, sporulation in direct contact with host cell cytoplasm ..... Unikaryon
- Parasites of spiders, spores in a vacuole in the cytoplasm of the host cell ..... Oligosporidium

67. Each sporont forms 6-8 spores ..... Geusia[4)]
- The sporont develops into an elongated plasmodium which produces a larger number of spores..Perezia

Comments

1) The description is diffuse and difficult to interpret. Exospore secretions of similar appearance occur within the genus Ameson. It is possible that we are dealing with a mixed infection.

2) The characters of Stempellia are based on Desportes' (1976) investigation of S. mutabilis which presently is the only species of the genus Stempellia s. str. They do not apply to Stempellia s. lat.

3) The genus is insufficiently known and the description does not provide any characters that definitely distinguishes it from Gurleya.
4) The genus is insufficiently described, characters uncertain.
5) G. habrodesmi Loubès, Maurand, Gasc & Bouix, 1976, was described from a polypod, but it is obvious that this species does not belong to the genus Glugea.
6) Two spore formation sequences occur among these genera. At present it is not possible to distinguish free spores of these genera from each other. Both genera form octospores in larvae and these can easily be identified with the identification key.
7) E. flavescens Weiser & Purrini, 1980, was described from Collembola. The authors state that this is a preliminary placing.
8) This genus is most probably not valid.

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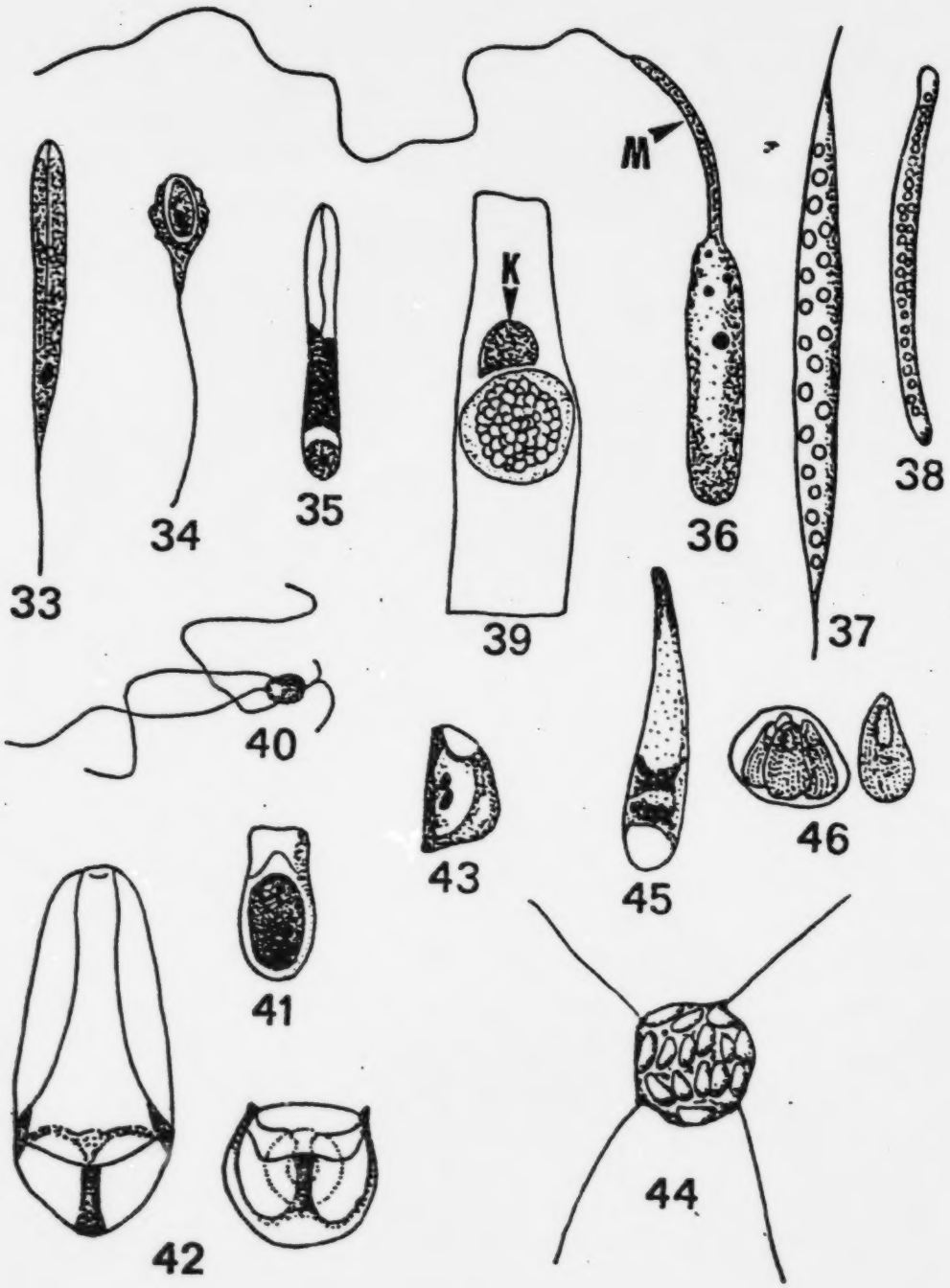
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Fig. 33. Spore of Jirovecia caudata with a tail-like extension (from Léger & Hesse, 1916).

Fig. 34. Spore of Caudospora simulii with tail-like extension (from Weiser, 1946).

Fig. 35. Spore of Cougourdella magna with enlarged posterior end (from Hesse, 1935).

Fig. 36. Spore of Mrazekia argoisi with extruded polar filament (from Léger & Hesse, 1916).

Fig. 37. Pansporoblast of Amphiacantha longa (from Caullery & Mesnil, 1914).

Fig. 38. Pansporoblast of Amphiamblys capitellidis (from Caullery & Mesnil, 1914).

Fig. 39. The pansporoblast of Chytridiopsis socius develops in the proximity of the host cell nucleus (from Schneider, 1884).

Fig. 40. Spore of Inodosporus spraguei with threadlike appendages (from Overstreet & Weidner, 1974).

Fig. 41. Spore of Parathelohania legeri with collar (from Kudo, 1924)

Fig. 42. Spores of Weiseria laurenti seen from the side and from one pole, respectively (from Doby &Saguez, 1964).

Fig. 43. Flattened spore of Golbergia spinosa (from Weiser, 1977).

Fig. 44. Pansporoblast of Trichoduboscqia epeori with thread-like appendages (from Léger, 1926).

Fig. 45. Pansporoblast and spore of Gurleya tetraspora (from Doflein, 1898).

TABLE OF MICROSPORIDIA GENERA

Explanations: Presence is marked with " + ", absence with " - ". In the column for 2 sporogonies "?" denotes that one sporogony is known, but it is suspected that one more type occurs. In the column for polar filament "?" denotes that polar filament is present, but it is not known whether it is aniso- or isofilar. In microsporidia with two different life cycles, a difference in characters is reported on either side of /. A large, irregular number is denoted by "n". Spore shape: O - oval, P - pyriform, R - spherical, S - rod-shaped. Polar filament: A - anisofilar, I - isofilar, M - manubrium.

	No. of nuclei: meront	sporont	spore	no. of sporoblasts	spore shape	micro + macrospores	2 sporogony sequences	pansporoblast	spores/pansporoblast	polar filament	examined in EM	xenoma	comments	Genus described by
Agmasoma	.		1/	8/	O P	+ ? +/		8/		A/	+ -			Hazard & Oldacre, 1975
Amblyospora	2	2	1/2	8/n	O	+ + +'-		8/-		A/I	+ -			Hazard & Oldacre, 1975
Ameson		2	2	n	O	- - -		-		I	+ -	-	1	Sprague, 1977
Amphiacantha	-	1	1	n	R	- - +		n		M	-	-	2, 3	Caullery & Mesnil, 1914
Amphiamblys	-	1	1	n	P	- - +		n		M	-	-	2, 3	Caullery & Mesnil, 1914
Aurospora		1/2	/2	n/	P	- + +/-		n/-		I	+	-	1	Weiser & Purrini, 1980
Baculea		1	1	2-4	S	- - -		-		M	+ -	-	25	Loubès & Alibaréh, 1978
Barvaldia	1	1	1	2	O	-	+	2		I	+ -	-	4, 5	Larsson, 1981
Baronella	1/2	1/2	1/2	8/2	O	- +	+/-	8/-		I	+ -	-		Jouvenaz & Hazard, 1978
Barbea	-	1	1	n	R	- -	-	-		I	+ +	-	2, 6	Sprague, 1977
Buxtehudea	-	1	1	n	R	- -	-	-		I	+ -	-		Larsson, 1980
Caudospora	1-2	2	2	8	O	- -	-	-		I	+	-	7	Weiser, 1946
Chapmanium			1/	8/	P	+ ? +/		8/		A/I	+ -	-		Hazard & Oldacre, 1975
Chytridiopsis	-		1	n	R	-	+-	n/		I	+ -	-	6, 8, 13	Schneider, 1884
Cougourdella	1?	1?	1	2?	R	- -	-	-		?	-	-	9	Hesse, 1935
Cryptosporina			1	8/	P	- ? +/		8/		I	+ -	-	10	Hazard & Oldacre, 1975
Culicospora	2	1	1	4,8,16	P	+ -	-	-		I	+ -	-		Weiser, 1977
Culicosporella			2	3-6	P	- -	-	-		I	+ -	-		Weiser, 1977
Duboscqia	1	1	1	16	S	+ -	+	16		?	+	-		Pérez, 1908
Encephalitozoon	1	1	1	2	O	- -				I	+			Levaditi, Nicolau & Schoen, 1923
Gousia		2	1	6-8	O	- -	-	-		I	+ -	-		Pühl & Korn, 1979
Gluea	1	1	1	2	O			-		I	+ +	-		Thélohan, 1891
Golbergia			2			- -				?			11	Weiser, 1977
Gurleya	1	1	1	4	O P	- -	+	4		I	+ -	-		Doflein, 1898
Haemodis	2	2/1	2/1	2?/2-16	P	- +	-	-		A/I	+ -	-		Weiser, 1977

	No. of nuclei: meront	sporont	spore	no. of sporoblasts	spore shape	micro + macrospores	2 sporogony sequences	pansporoblast	spores/pansporoblast	polar filament examined in EM	xenoma	comments	Genus described by
Nelmichia	2	2	1/	8/	S	- ? +/		8/	I	+ -			Larsson, 1982
Pessea	2	2	1-2	2	R	- - +		n	I	+ -		6, 8, 25	Ormières & Sprague, 1973
Heterospora				8(16)	O	+	+	8(16)	?				Schubert, 1969
Hyalinocysta			1	8/	P	- ? +/		8/	4/	+ -		12	Hazard & Oldacre, 1975
Ichthyosporidium		2	2	2	C	- -	-	-	I	+ +			Caullery & Mesnil, 1907
Inodosporus				8/	P	- ? +/		2/	I	+ -		11	Overstreet & Weisner, 1974
Issia			2	2	O	+ - +		2	?	-		4	Weiser, 1977
Jirovecia			1		S	- - -	-	-	M?	+		7	Weiser, 1977
Jiroveciana	-		1	n	R	- - -	-	-	?	-			Larsson, 1980
Matchinkovella	-	1	1	n	R		+	n	M	+		2, 3	Caullery & Mesnil, 1897
Nitoplistophora				n	P	- - +		n	?	-		14	Codreanu, 1966
Nosakia	2	2	2	2	S	- - -	-	-	M	+ +		2	Léger & Hesse, 1916
Nosperoxia	2	2?	1?	2	O	-	+	2	?	-		4	Issi & Voronin, 1979
Nosema	2	2	2	2	O			-	I	+ -			Naegeli, 1857
Nosemoides	1	1	1	n	S S	+ -	-	-	I	+ -			Vinckier, 1975
Octosporea			2	8	S	- +	+	8	I	+ -			Wu, 1931
Oligosporidium	1	1	1	2	O	- - -		-	I	+ -			Codreanu-Balcescu, Codreanu & Tractuar, 1981
Ormierella	2		1	8	S	-	+	8	M I	+ -		27	Alvarez, Bonix & Hunter, 1977
Parathelohania	2	2	1/2	8/n	O		+ +/-	2/-	8/I	+ -		15	Codreanu, 1966
Pegmatospora			1/	8/	O	- ? +/		4/	I	+ -			Hazard & Oldacre, 1975
Perezia	2	2	1	n	O	- - -		-	I	+ -			Léger & Duboscq, 1909
Pilosporella				8/	E	- ? +/		1/	I	+ -			Hazard & Oldacre, 1975
Pleistophora	1	1	1-2	n,8	O	+ + +		n,8	I	+ -			Canning, 1983
Pseudoloisiophora		2	2	n	O		+	n	I	+ -		16	Sprague, 1977
Tuzetia			1-2	4	S	- - +		4	I	+ -		17	Hesse, 1935
Sprague			1	n	O	- - -	-	-	?	+ +		18	Weissenberg, 1976
Steinhausia	-	1	1		S	- - -	-	-	I	+ -		6, 8, 19, 20	Sprague, Ormières & Hunter, 1972
Stempellia	1	1	1	4,8	O P	+ +	+/-	4/-	I	+ +			Léger & Hesse, 1910
Systenostrema			1	8/	O P	- ? +/		8/	4/	+ -			Hazard & Oldacre, 1975
Telomyxa	1		1	2	O	- - +		2	I	+ -		21	Léger & Hesse, 1910
Trelonania	2	2	1/2	8/n	O P	+ +	+/-	2/-	I	+ -			Bonnegoy, 1932
Toxoluges	2	2	1/	8/	S	+ ? +/		8/	I	+ -		22	Léger & Hesse, 1924
Trichothoracia		1		16	O	- - +		16	?	-		23	Léger, 1926
Tuzetia	1	1	1	3-4	O P	- - +		1	I	+ -			Naerssen, Pize, Pomrich & Michel, 1971
Urlivora	1	1	1	2	P	- - -	-	-	I	+ -			Canning, Lai & Lie, 1974
Vairimorpha	/2	2	1/2	8/2	C	- + +/		2/	?	-			Pilley, 1976
Vavraia		1		32	O	+ - +		32	?				Weiser, 1977
Weiseria		1	2	16-25	O	- - +		16-32	?	-		24	Doby & Savoie, 1966

COMMENTS TO TABLE

1 epispore secretion present
2 polaroplast absent
3 endospore absent
4 spores attached in pairs
5 two-layered pansporoblast membrane with fibrillar inner layer
6 polaroplast is rudimentary or absent
7 spore with tail-like appendage
8 endospore is rudimentary or absent
9 posterior pole of spore enlarged
10 pansporoblast with large crystals that nearly conceal the spores
11 spore shape flattened, unique
12 pansporoblast without inclusions, clear
13 spore with 4-5 appendages
14 spindle-shaped pansporoblast with thread-like, elongated corners
15 octospores with posterior end fanned out like a collar
16 pansporoblast that branches out like fingers in the cytoplasm
17 drop-shaped spores with elongated point
18 xenoma resembling a cluster of grapes
19 develops in the proximity of the host cell nucleus, often in a bowl-like structure
20 exterior of polar filament covered by a honey-comb layer
21 spores enclosed in a massive, oval pansporoblast
22 spores "c"-shaped or spiral-twisted
23 pansporoblast with needle-like appendages
24 spores with ridges or collar
25 pansporoblast with a mosaic of polygonal plates which have disc-shaped appendages
26 polaroplast is small, does not surround the polar filament
27 anterior end of spore has 2 polar rings

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ADDENDUM

After the manuscript was completed two more valid genera have been described. The genus Loma Morrison & Sprague, 1981 (Journal of Fish Diseases 4: 15-32) parasitizes the gills of cod and salmon fishes. All stages are mononuclear. Sporogony is disporoblastic, without pansporoblast. During spore formation the spores are surrounded, singly or in pairs, by a vacuole. Spores are oval and of uniform size. Polar filament is isofilar. The parasite forms xenomas with cell hypertrophy. The description is based on electron microscopic examination. Pleistophora simulii (Fig. 24) has been removed from its previous genus and is now the type species for a new genus, Polydispyrenia, Canning & Hazard, 1982 (Journal of Protozoology, 29: 39-40). The diplokaryotic meront develops into a plasmodium with diplokarya, which is divided into smaller plasmodia. Each diplokaryon undergoes meiosis and produces 8 haploid nuclei, each one of which produces a mononuclear sporoblast. Spores are haploid, mononuclear. The shape is oval and the size uniform. Sporogony takes place inside a pansporoblast membrane. The number of spores in the pansporoblast depends on the number of diplokarya in the sporogonial plasmodium. Pansporoblast with tubular inclusions. Polar filament is isofilar. Ultra-structural characters are known. It is probable that diploid, infectious spores are also formed. The genus Pseudothelohania Codreanu & Codreanu-Balcescu, 1982 (Journal of Protozoology, 29:301) was described without any information about which species belong to it and is therefore not valid.